

## Mini Review

# Protein Glycation: An Old Villain is Shedding Secrets

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## Abstract

In the last two decades, interest in the pathogenic nature of glycated proteins has spread to enmesh numerous metabolic, autoimmune and neurological disorders, tying together several confounding aspects of disease etiology. From diabetes, arthritis and lupus to multiple sclerosis, Alzheimer's and Parkinson's diseases, the glycation / inflammation paradigm offers significant insight, exposes new drug targets and treatment options, and may even lay foundations for long-awaited breakthroughs.

**Keywords:** Glycation; Advanced glycation end-products (AGE); Inflammation; Autoimmune; Diabetes; Lupus; NPSLE; Neurodegeneration

## Introduction

Many maladies continue to defy medical cures, but usually the battle lines are clear. Infectious diseases and cancer, for example, represent cellular struggles of good and evil. Such clarity always seems to give us the leverage and will to prevail, but where does that leave chronic metabolic, inflammatory and neuropathic syndromes? They offer no 'good vs. evil' dichotomy. Such afflictions are like civil wars, where our own hard-working physiological resources are subverted into internecine paths of self destruction.

Like any bewildering conflict, the central, crucial question is simply, *why?*

Why do skin, intestine, cartilage, spine or brain inflame when there is no infection to kill?

Glimmers of an answer emerged in 1997, with an influential clinical report by Koschinsky [1] that began to transform our understanding of diabetes mellitus. Pushing past preconceptions of a metabolic disease arising from a semi-idiopathic set of lifestyle risk factors, the paper zeroed in on diabetic correlations with chronic serum elevation of abnormally modified proteins and lipids called Advanced Glycation End-products (AGEs).

AGEs were (and still are) a common feature of modern diets (they are byproducts of high temperature cooking, are used as food additives to enhance flavor and texture), yet the Koschinsky study boldly labelled them as '*glycotoxins*', based on *in vivo* evidence that dietarily

consumed AGE proteins might convert physiological fibronectin into a pathogenic glycated form implicated in renal and vascular lesions [2]. The paper contrasted the outcomes of an elevated-AGE diet versus a more healthy alternative, and profiled the time-varied glycation of fibronectin across cohorts of diabetics and non-diabetics, finding clear correspondence between AGE intake and glycation prevalence in physiological proteins. A healthy diet generally lowered serum glycated fibronectin levels for diabetics and non-diabetics alike but, interestingly, while the AGE diet produced elevated glycations in both non-diabetics and diabetics, the latter cohort required an alarmingly long time to clear the non-physiological protein.

This profound finding suggests dangerous a feedback spiral, implying that modern diet embraces chemicals that promote metabolic dysfunction and inflict elevated harm on people with already weakened metabolic function.

The subsequent two decades have broadened the picture of how AGE compounds, glycated proteins, and chronic illness reinforce each other. Further studies have validated the original Koschinsky conclusions and, indeed shown them to be the tip of an iceberg. With known implications far beyond the renal and vascular lesions of diabetes, glycation now reaches into the etiology of many of the world's prevalent and debilitating metabolic, autoimmune, and neuropathic syndromes.

However sobering this insight may be, it is highly encouraging. The more we understand a threat, the greater our chances of developing meaningful therapeutic countermeasures. To this end, let us briefly review an emerging consensus on structure, mechanism, and biochemistry of glycated proteins.

## Advanced Glycation End-products

The precise phrasing of standard textbook definitions of 'AGE' varies, but virtually all emphasize the term 'non-enzymatic'. Thus, beyond the standardized slate of physiological post-translational modifications, proteins may be modified via sporadic covalent adduction (and potential cross-linkage) of carbohydrates such as glucose, galactose, glyoxal, methylglyoxal, fructose, etc. Whereas physiological glycosylation occurs on hydroxyl groups (serine and threonine amino-

**Citation:** Lushington GH. Protein Glycation: An Old Villain is Shedding Secrets. *Ann Integr Mol Med.* 2019; 1(1): 1001.

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**Publisher Name:** Medtext Publications LLC

**Manuscript compiled:** March 13<sup>th</sup>, 2019

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acid side chains), phosphate oxygens (phospholipids) or amide nitrogens (asparagine side chain), glycation targets free amines (lysine chain chain or phosphadidylethanolamine) or guanidines (arginine side chain) [3]. For proteins, this distinction has immense functional consequences.

Physiological modifications made to serine, threonine and asparagine residues only modestly modulate protein structure for the simple reason that most Ser, Thr and Asn groups are positioned so as to project a polar side chain out into a polar medium (e.g., plasma) to mediate protein solubility. Adding glycans to such residues increases bulk that may stiffen the protein somewhat, but do not affect net polarity and rarely induce only slight conformational perturbations. Lysine and arginine, however, often form salt bridges responsible for protein tertiary stabilization or quaternary coupling. Neutral glycation of an erstwhile cationic Lys or Arg side chain is thus likely to either destabilize protein folds, or alter protein-protein interaction landscapes. Furthermore, modification of lysine and arginine residues tends to block many normal proteolytic mechanisms, leading to much greater metabolic persistence of glycated proteins.

Consider the aforementioned case of fibronectin – a structural support protein that forms matrices to foster cell growth and differentiation, intercellular adhesion, and wound healing [4]. Depending on the residues targeted by glycation, any of these functions can be partially or fully disrupted, resulting in tissue lesions found in diabetes mellitus [5] and other maladies.

Many proteins other than fibronectin may become glycated, and may do so in ways that do not require dietary consumption of exogenous AGE molecules. Indeed, although ingesting AGEs from dietary may help to initiate glycation pathologies, a great proportion of the glycotoxins present in serum are manufactured in situ, with endogenous protein glycation occurring spontaneously during the release of reactive oxygen species upon exposure to ultraviolet radiation [6] and within inflamed tissue [7].

## Brief Survey of AGE Pathology

Recent research has shed light on the role of glycation in a diverse array of pathologies [8]. AGE molecules have been found to significantly influence the onset of multiple aspects of diabetes, including not only the aforementioned nephropathic lesions [1], but also related cardiovascular complications, and diabetic retinopathies and neuropathies [9]. The slate of associated pathogenic glycated proteins includes fibronectin, hemoglobin, Human Serum Albumin (HSA), various immunoglobulins, and the apolipoproteins ApoA1 and ApoB [10,11].

Beyond diabetes, AGE molecules have demonstrated a propensity for spurring inflammation while resisting quick metabolic clearance, and thus have been identified as active components in numerous autoimmune disorders. In rheumatoid arthritis, glycated immunoglobulin G (IgG) distribution correlates strongly with tissue inflammation [12,13]. Pathologically glycated IgG appears also to be the critical immune trigger for bouts of inflammatory bowel diseases such as Crohn's and colitis [14]. The polyphasic immunopathologies associated with systemic lupus erythematosus (SLE) suggest complex causation, which may correspond to chronic distributions of glycated IgG [15], glycated beta-2-microglobulin (B2M) [16] and glycated HSA [17], associated with anomalies in the classical complement immune pathway. In particular, many (or most) SLE patients are deficient in Complement C1q, which primarily targets glycosylated proteins on

pathogens (e.g., on microbes [18] and cancer cells [19]), but is now also understood to be crucial for clearance of glycated proteins [20].

As saying the complex pathology of SLE has shed light on broader implications of autoimmune dysfunction. Glycation-induced inflammation has been known (e.g., glycated hemoglobin in retinopathies [21]) or suspected (e.g., glycated HSA in neuralgias [22]) to cause a variety of peripheral neurological syndromes, but we are beginning to grasp how such effects also extend into the central nervous system (CNS). Indeed, a majority of SLE patients experience CNS an array of different CNS disorders [23], collectively known as neuropsychiatric lupus (NPSLE). This peripheral to central cross-talk necessitates a sobering recalibration of long-held assumptions that the blood brain barrier (BBB) could reliably defend the brain against spread of peripheral disorders.

Not surprisingly, AGE proteins play a role in BBB compromise in NPSLE patients. Glycated proteins resident in the endothelia of tight vascular junctions comprising the BBB spurs inflammation and also, more critically, stimulate excessive expression of the vascular regulating protein VEGF [24]. This leads to vascular malformations, resulting in endothelial leakage [25] that fosters an effective transfection of the peripheral autoimmune battle into the CNS. Specifically, both the immunological hunters (B-cells [26] and CD8+ lymphocytes [27]) and quarry (glycated HSA, B2M and apolipoproteins) enter the brain, wherein the glycated antigens embed in neuronal, dendritic and axonal surfaces where they incite inflammatory attack.

NPSLE pathology presents various symptoms that, depending on pathogen distribution across the brain, may entail seizures, psychoses, motor disruptions, variable consciousness, and cognitive deficits. These NPSLE symptoms mirror many found in CNS-only neuropathies, which leads us to recognize the newest emerging front in glycation pathology research – the apparent role of AGE compounds in neurodegenerative, neuromuscular, and neuropsychiatric disorders.

Molecular correspondence between NPSLE and other neuropathies is profound. Elevated presence of glycated B2M, a key SLE antigen, is also a strong biomarker for Alzheimer's and Parkinson's diseases [28], and also correlates with schizophrenia and various forms of depression [29]. The  $\epsilon 4$  allele of apolipoprotein ApoE is a glycation-susceptible protein known not only as an NPSLE risk factor, but also as one of the strongest biomarkers for Alzheimer's [30], Parkinson's [31], multiple sclerosis [32] and other neuropathologies. Additionally, there is an emerging suspicion that neurodegeneration is exacerbated by CNS inflammatory production of metabolically stable glycated isoforms of many key pathogenic proteins such as beta amyloid [33], tau [34], alpha synuclein [35], huntingtin [36] and perhaps TDP-43 [37].

## Therapeutic Implications

On one hand, the spectre of our widespread dietary exposure to a class of chemicals that may incite self-perpetuating cycles of protein corruption and inflammatory response is somewhat terrifying. Conversely, though, there is reassurance that as we come to fully characterize the threat of glycation-based xenobiochemistry, we may open many new doors to medicinal intervention and beneficial lifestyle adjustments. We may also take heart in insight arising from what began as a thread within diabetes research may ultimately bear medical fruit in treating a diverse slate of other challenging diseases. In this vein, meaningful drug repurposing is already exploring therapeutic prospects toward a long prospective list of metabolic, inflammatory and

neurological disorders [38].

There is further comfort in an opportunity for redemption. Having identified the mechanisms by which harmful glycation / inflammation spirals begin, we can now hone our research in pursuit of corrective courses. We have evidence that modest lifestyle adjustments achieve statistically meaningful anti-glycation benefits [39], and reviews suggest vigorous efforts in conventional drug discovery [40], focusing on targets such as BDNF [41] and Nrf2/Keap [8]. Natural products investigations [42] may also prove especially rewarding, given preliminary anti-glycation indications from herbal extracts including (among others) curcumin, isoflavonoids and various common vitamins [43]. So, will these recent revelations into pathogenic glycated proteins resolve some of those physiological 'civil wars' that we know as metabolic, inflammatory and neuropathic syndromes? Answers will take time and effort but, perhaps, a glycotoxic sugar-coated cloud is finally beginning to lift.

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